



Identification of sources of *Salmonella* in watersheds and characterization of multiple antimicrobial resistant strains

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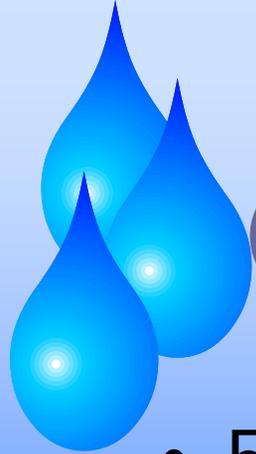
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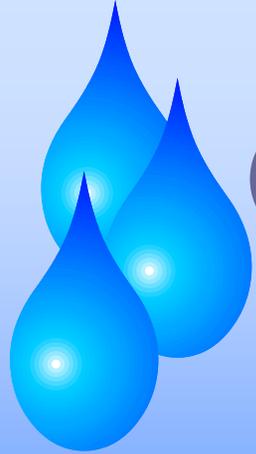
⁴Dept. of Environmental and Molecular Toxicology, NCSU





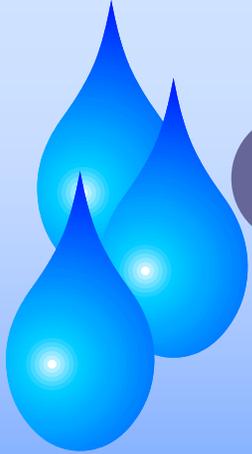
Background

- 56,000 acres of shellfish beds along coastal NC are closed due to elevated levels of bacteria
- Bacterial contamination are assumed to originate by human activities (farming, forestry, and urbanization)
- One of the densest swine production area

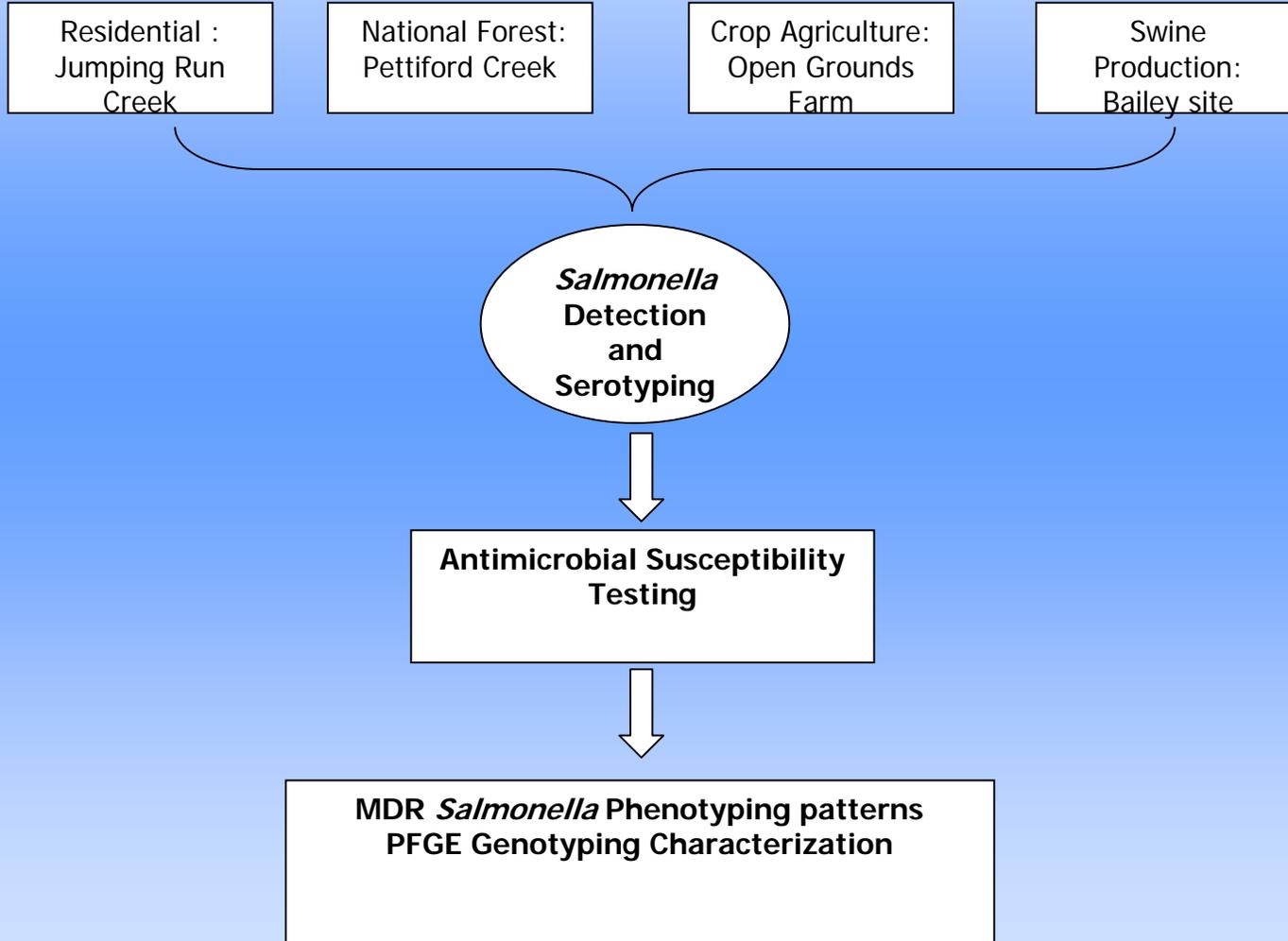


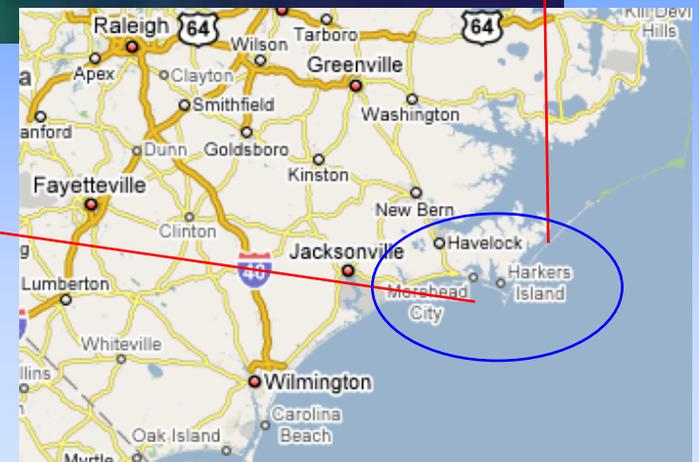
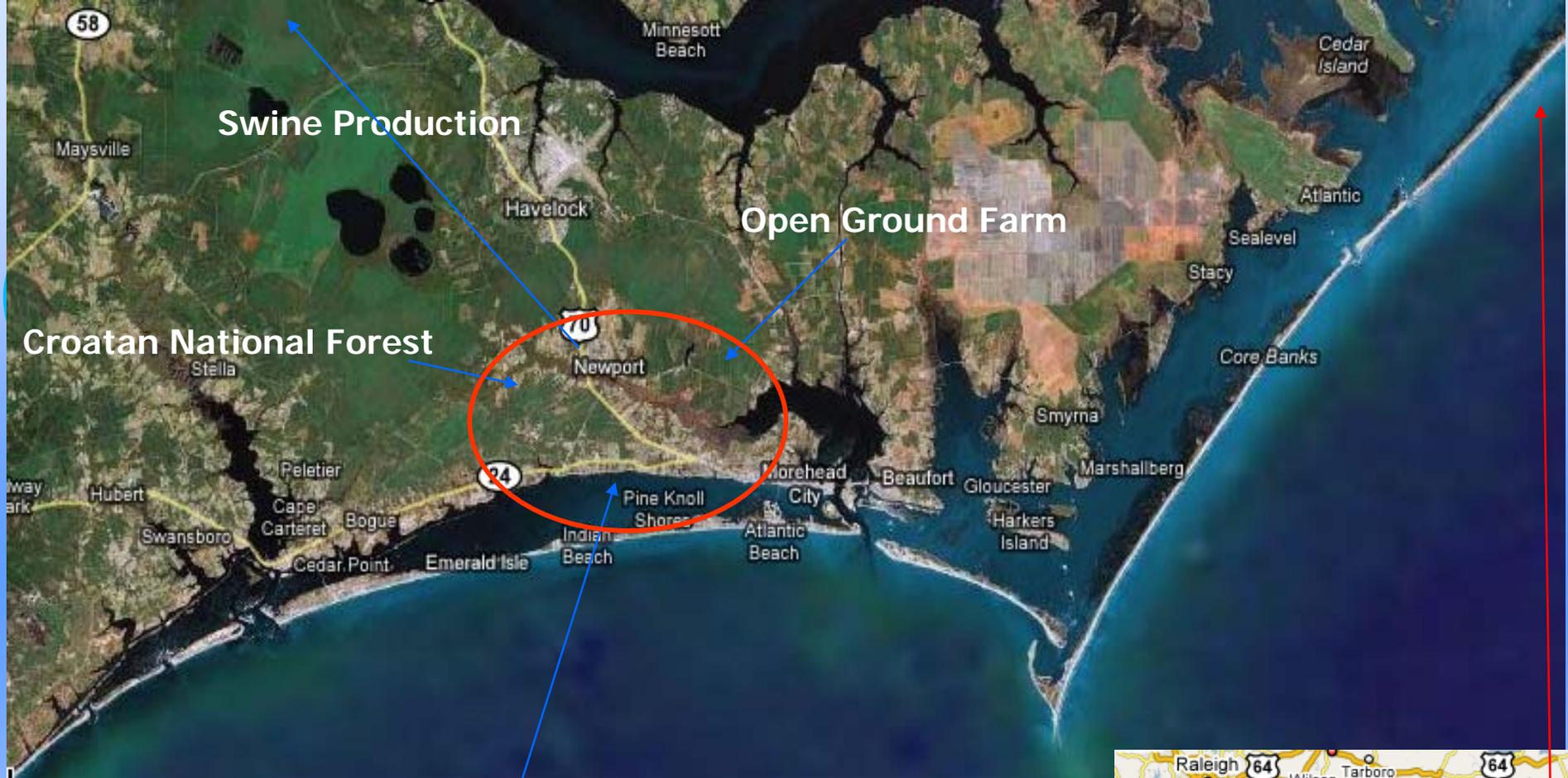
Objectives

- To identify *Salmonella* sources of water contamination
- To compare and understand antimicrobial resistance patterns and genotypes among environmental, animals and humans waste
- To expand knowledge based on MDR and diversity of *Salmonella spp.*



Project overview







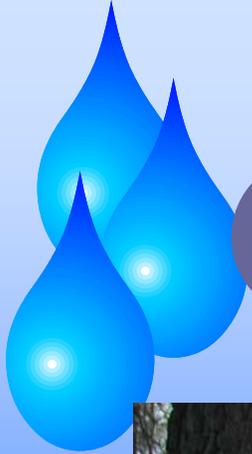
Pettiford Creek





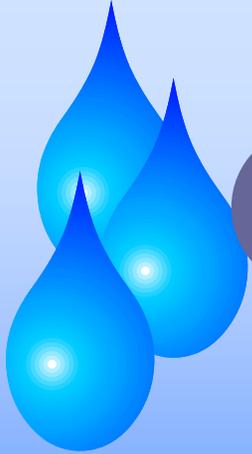
Open Ground Farm





Monitoring site





Microbiological culture



25 ml water sample+25 ml BPW

37° C ↓ **18-24 hrs**

100 µl Aliquot+10ml RV



42° C ↓ **18-24 hrs**

XLT-4 (Xylose Lysine Tergitol-4) Plate

37° C ↓ **18-24 hrs**



Biochemical Test

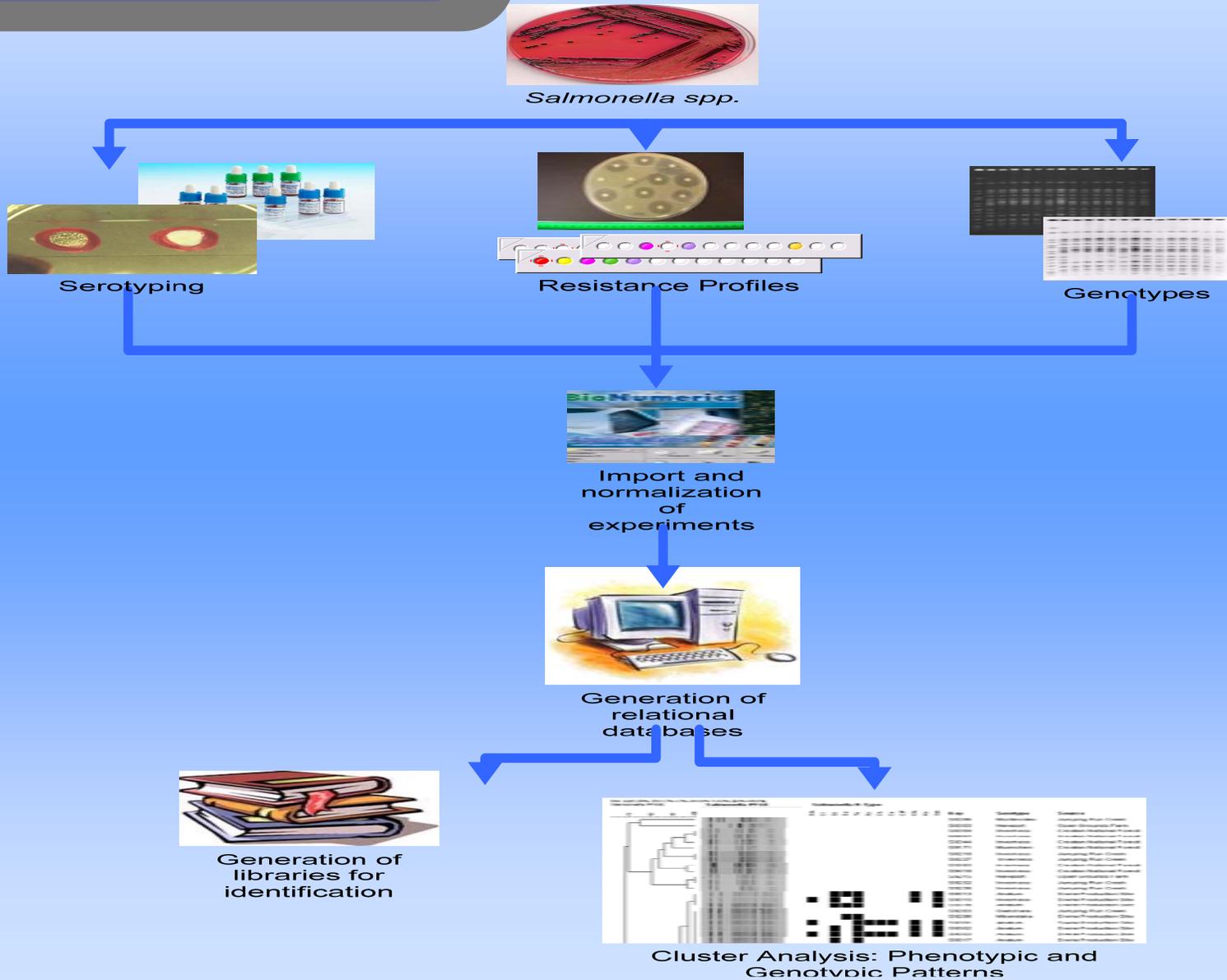
(TSI and Urea)

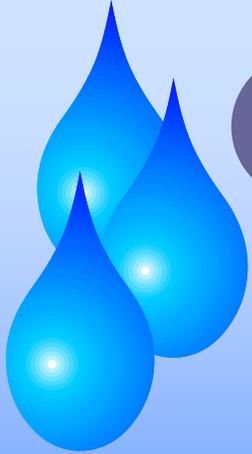
37° C ↓ **18-24 hrs**



Slide Agglutination Polyvalent Antisera

Methodology Flowcharts

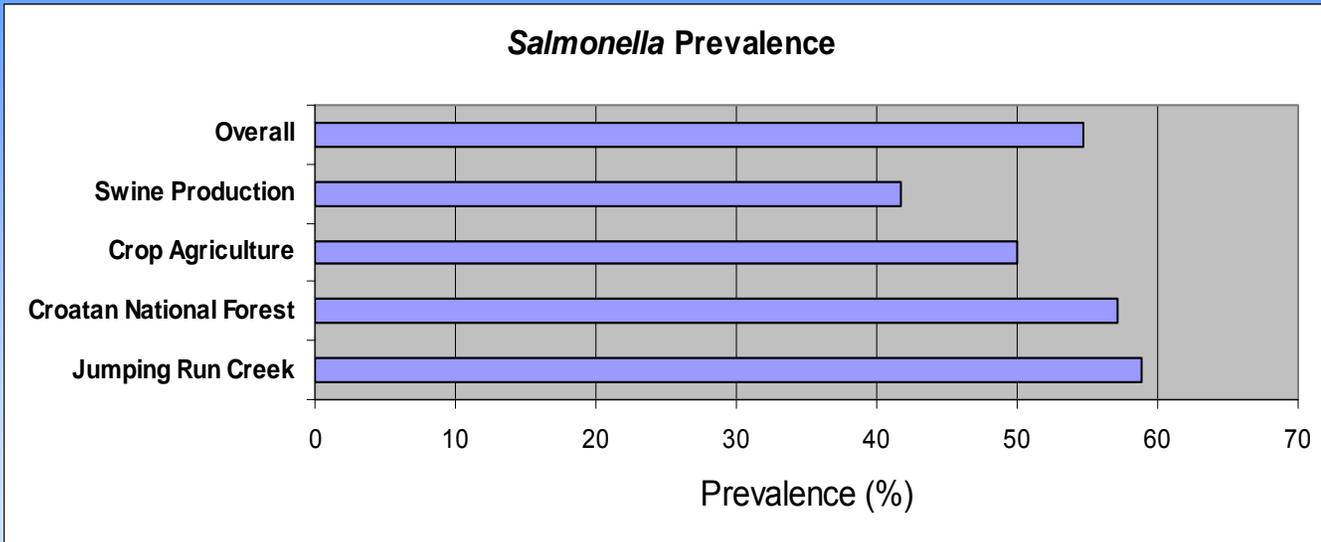




Results

Salmonella Prevalence

Water Source (n)	Prevalence (%)	95%CI
Jumping Run Creek (34)	58.8	41-76
Croatan National Forest (28)	57.1	38-77
Crop Agriculture (12)	50	17-83
Swine Production (12)	41.7	9-74
Overall (N=86)	54.7	44-65



Serotype Diversity

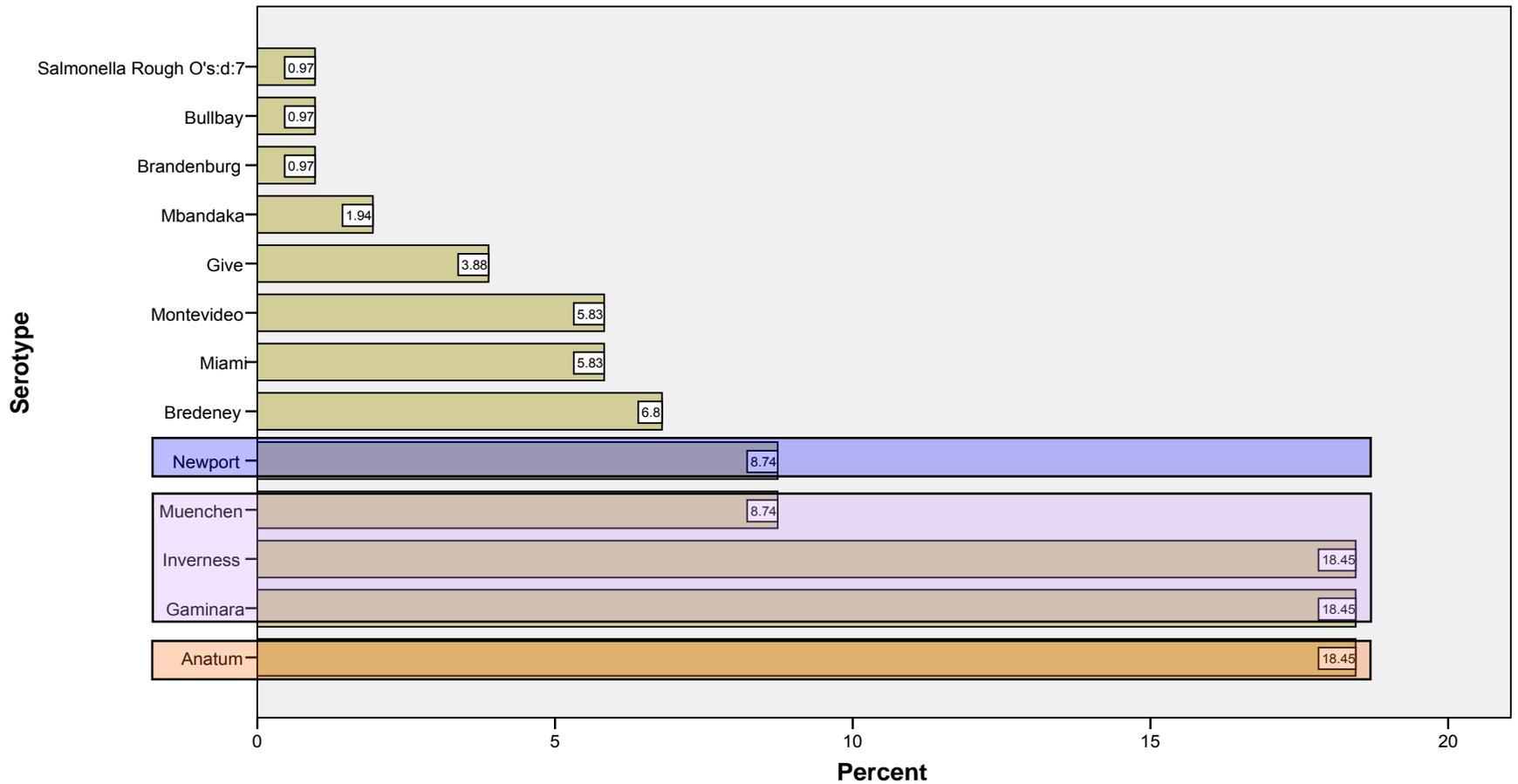
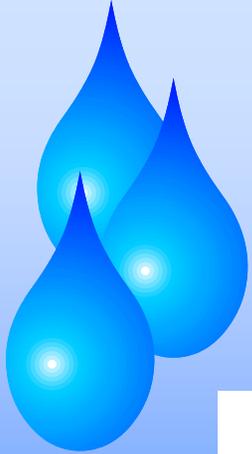




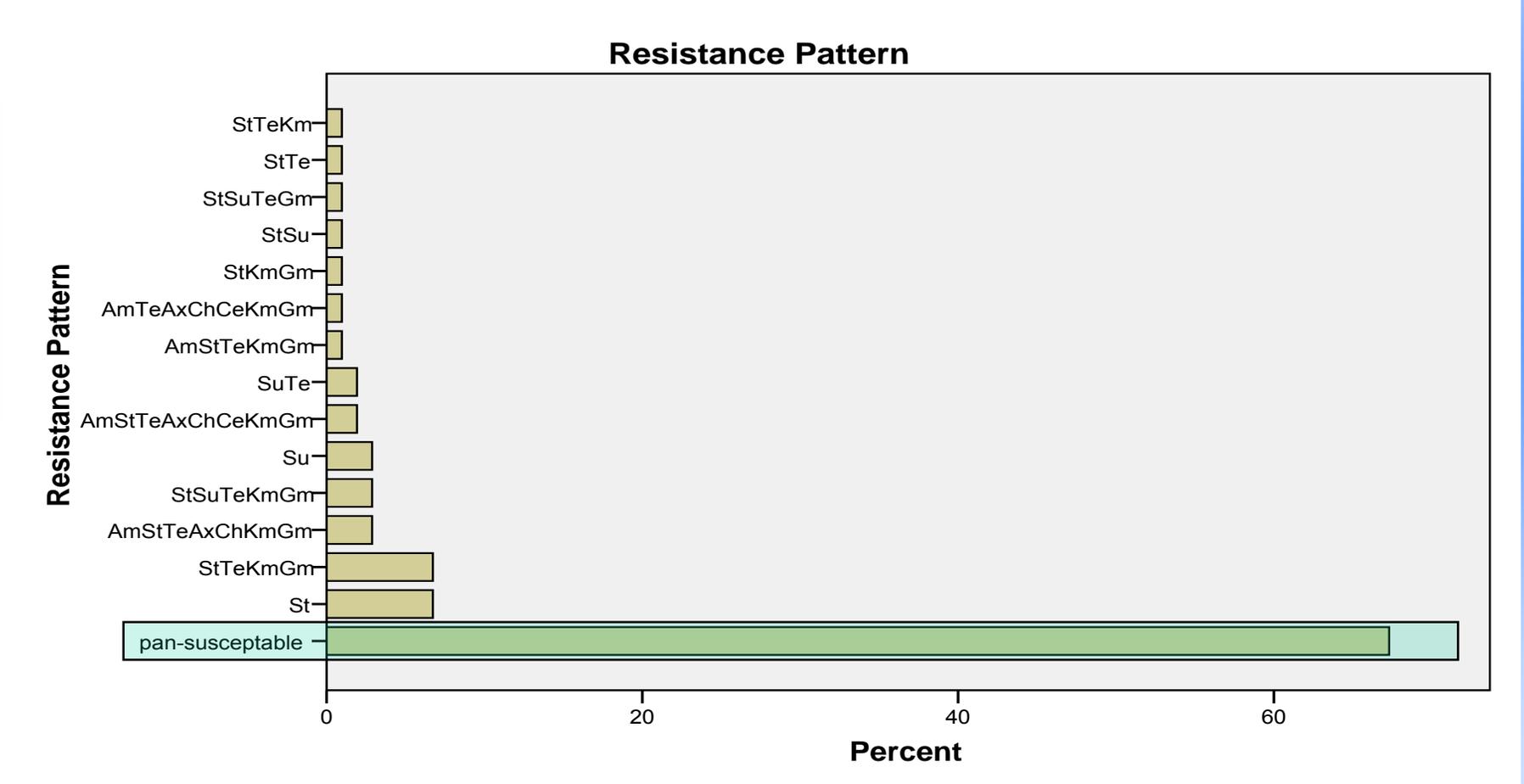
TABLE 1

The 30 most frequently reported Salmonella serotypes from Human sources reported to CDC in 2005

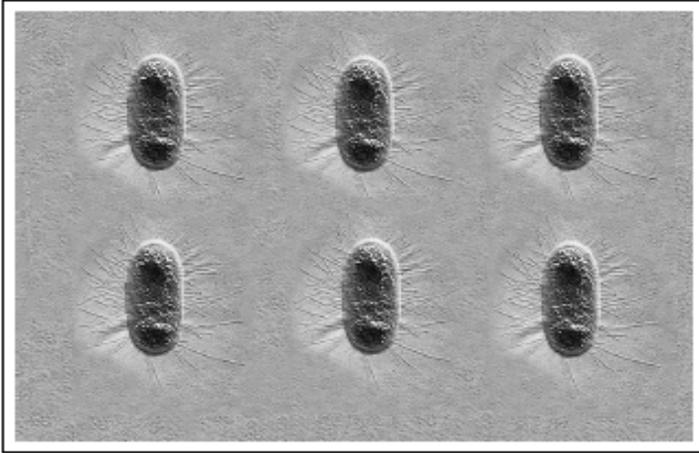
Human 2005			
Rank	Serotype	Reported	Percent
1	Typhimurium *	6982	19.3
2	Enteritidis	6730	18.6
3	Newport	3295	9.1
4	Heidelberg	1903	5.3
5	Javiana	1324	3.7
6	14,[5],12:i:-	822	2.3
7	Montevideo	809	2.2
8	Muenchen	733	2.0
9	Saintpaul	683	1.9
10	Braenderup	603	1.7



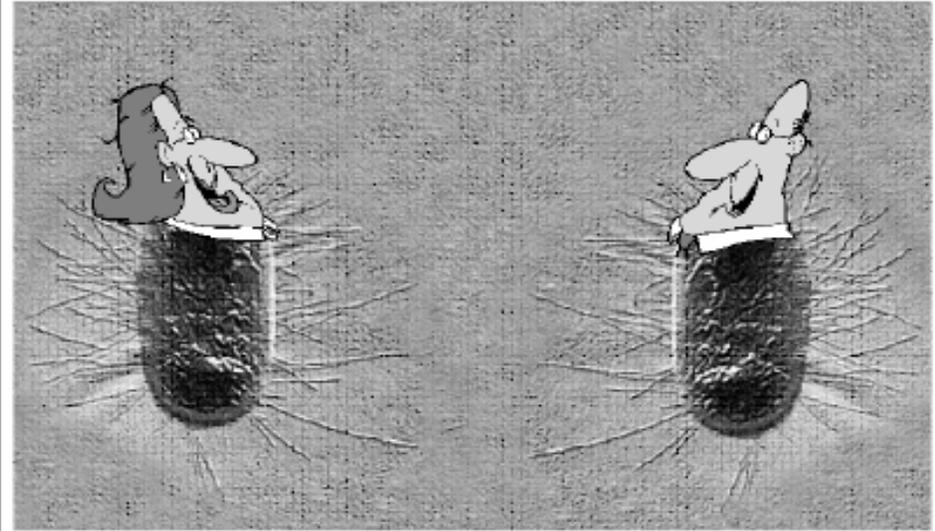
Resistance profile diversity



WHICH ONE OF THESE IS NOT LIKE THE OTHERS?

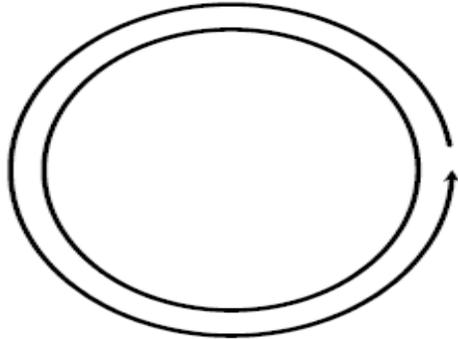


A QUESTION OF INTERRELATIONSHIP

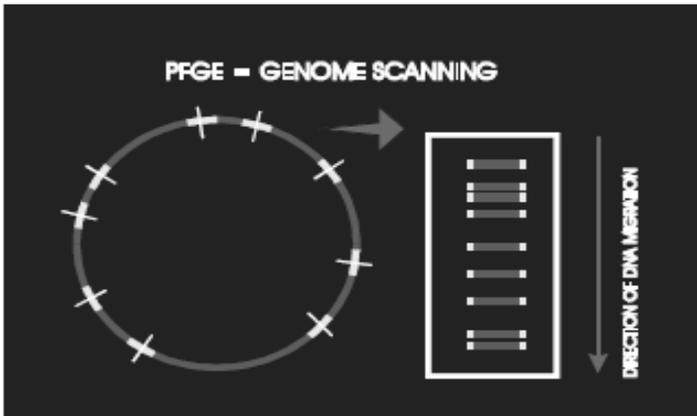


Pulse Field Gel Electrophoresis (PFGE)

PFGE MONITORS >90% OF THE CHROMOSOME



PFGE = GENOME SCANNING



Bacterial Suspension

Plug Mold

Mix with Agarose

Chemical Lysis and Washing

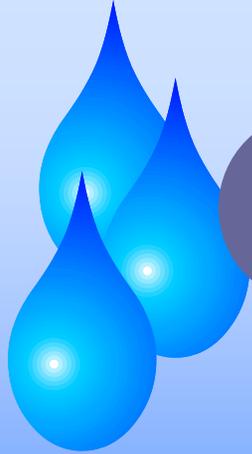
DNA in Plugs

Restriction Endonuclease

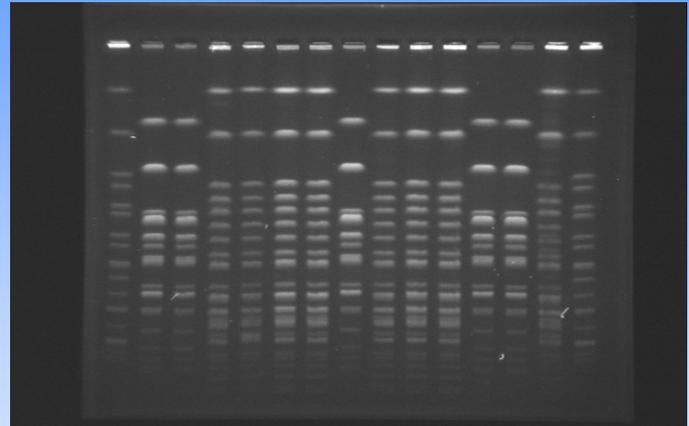
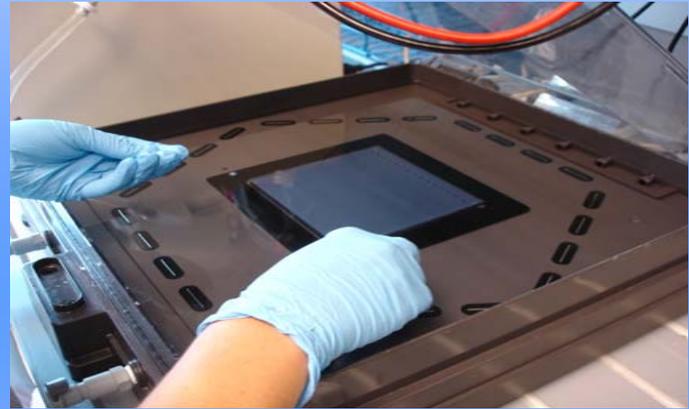
Electrophoresis (PFGE)

Documentation (capture gel image)

Data Analysis



PFGE Process



Similarity Measurements

PFGE Fingerprinting

Band Position	sampleA	sampleB
1	—	—
2	—	—
3	—	—
4		—
5		
6	—	—
7	—	—
8	—	
9	—	
10		

Index Value: Dice Similarity Index
 $S_d = 2a / (2a + b + c)$

		Sample A	
		yes	no
Sample B	yes	5	1
	no	2	2

		Sample A	
		yes	no
Sample B	yes	a	b
	no	c	d

$$S_d = \frac{2 \cdot 5}{(2 \cdot 5 + 1 + 2)}$$

$$= 0.76$$

Similarity Measurements

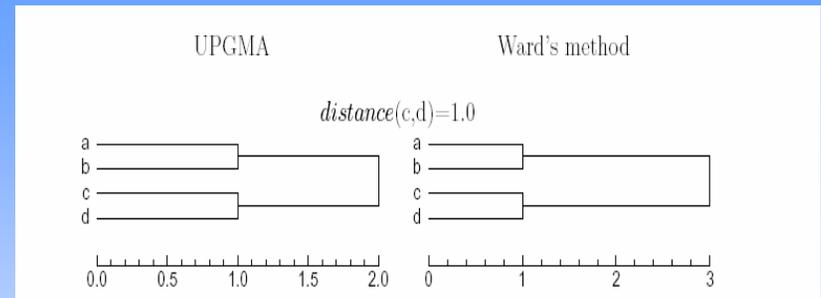
Fingerprinting Patterns

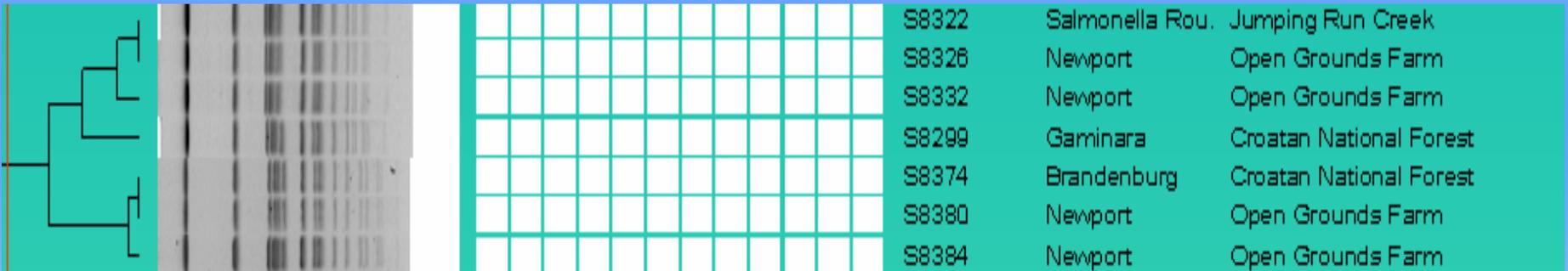
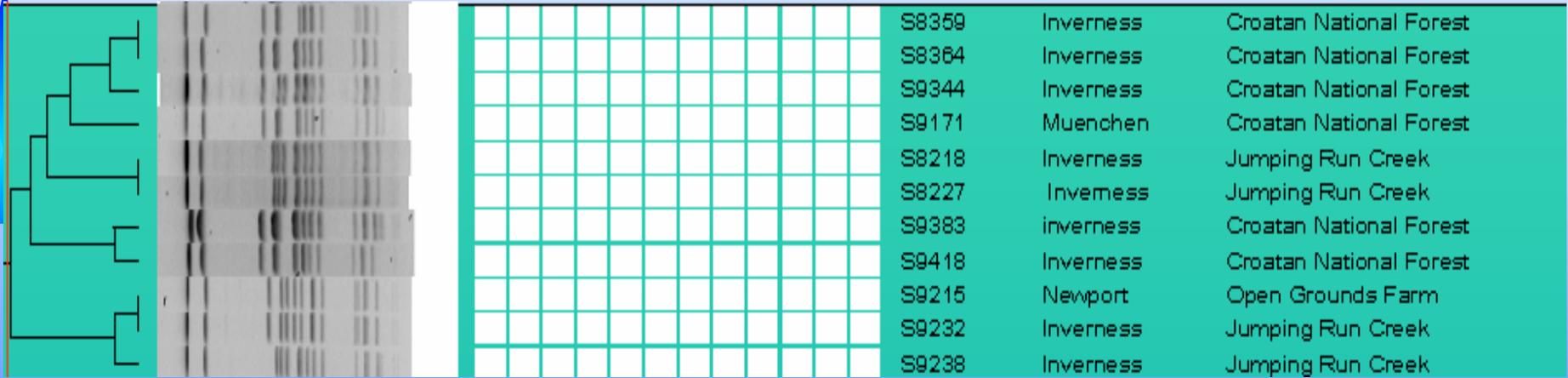
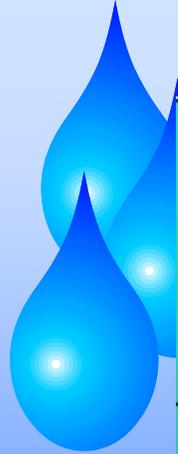
Band Position	sampleA	sampleB	SampleC	SampleD	sampleE
1	-	-	-	-	-
2	-	-	-	-	-
3	-	-	-	-	-
4		-	-	-	-
5			-	-	-
6	-	-	-	-	-
7	-	-	-	-	-
8	-		-	-	-
9	-			-	-
10			-	-	

Similarity Coefficient Matrix

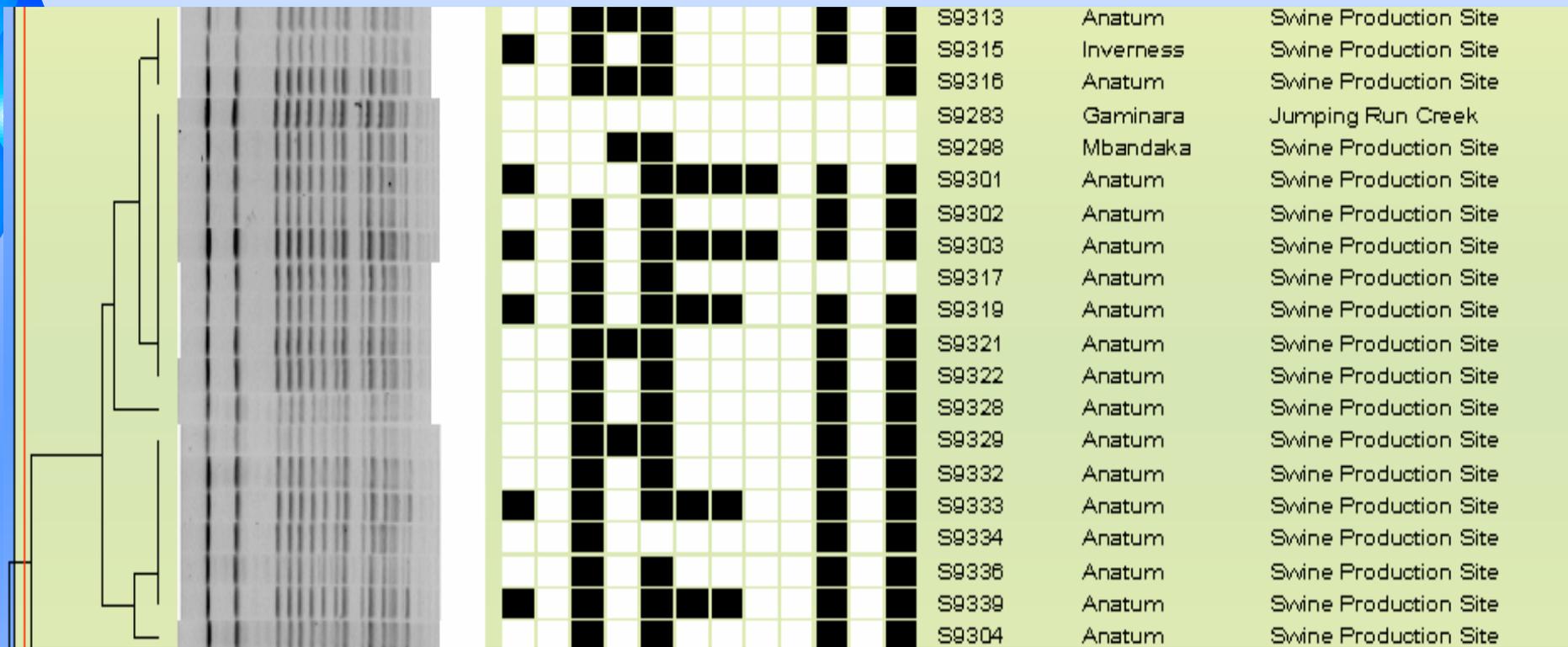
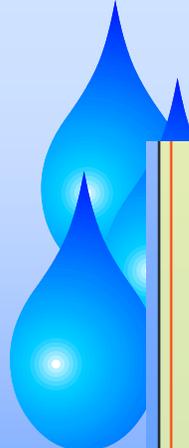
Sample	A	B	C	D	E
A	1.00	0.63	0.55	0.60	0.44
B		1.00	0.60	0.50	0.5
C			1.00	0.73	0.5
D				1.00	0.5
E					1.00

Cluster Analysis





PFGE and cluster analysis demonstrated the genetic clonality among *Salmonella* strains recovered from Croatan National Forest, Jumping Run Creek and Open Ground Farm



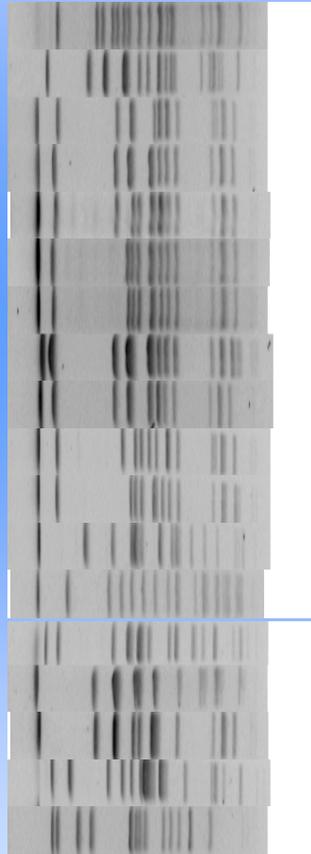
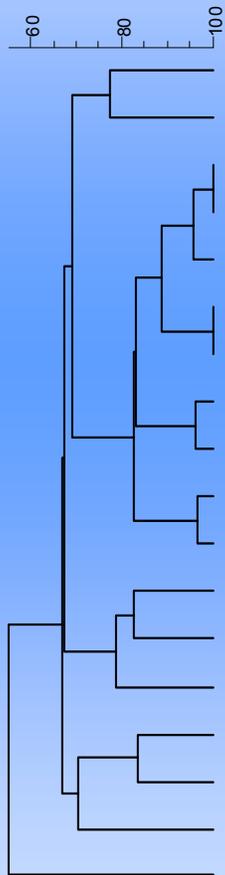
Swine strains were identical and grouped together

Dice (Opt:2.00%) (Tol 1.5%-1.5%) (H>0.0% S>0.0%) [0.0%-100.0%]

Salmonella PFGE

Salmonella PFGE

Salmonella R-Typ



Am Cl St Su Te Ax Ch Ce CIP Km An Gm

Key

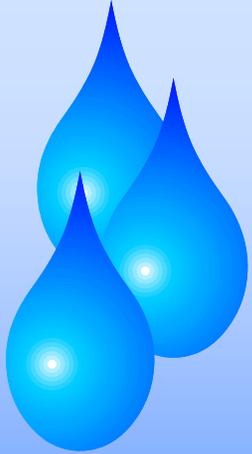
S8222
S8393
S8359
S8364
S9344
S8218
S8227
S9383
S9418
S9232
S9238
S9210
S9315
S9176
S9167
S9368
S9248
S8368

Serotype

Inverness
Inverness

Source

Jumping Run Creek
Croatan National Forest
Croatan National Forest
Croatan National Forest
Croatan National Forest
Jumping Run Creek
Jumping Run Creek
Croatan National Forest
Croatan National Forest
Jumping Run Creek
Jumping Run Creek
Open Grounds Farm
Swine Production Site
Croatan National Forest
Croatan National Forest
Jumping Run Creek
Jumping Run Creek
Croatan National Forest

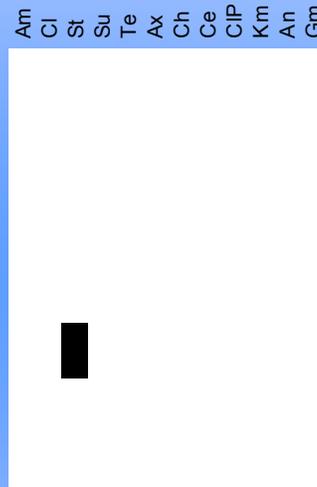
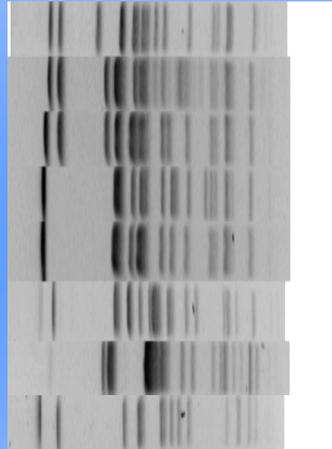
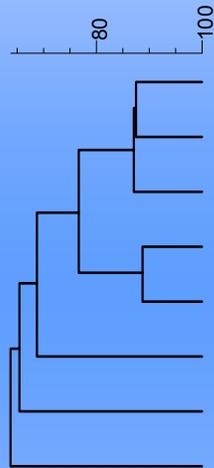


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Salmonella PFGE

Salmonella PFGE

Salmonella R-Type

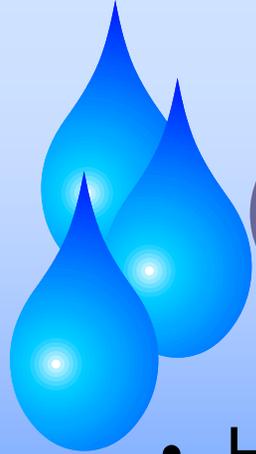


Key

Serotype

Source

S9365	Muenchen	Jumping Run Creek
S9397	Muenchen	Croatian National Forest
S9404	Muenchen	Croatian National Forest
S9409	Muenchen	Croatian National Forest
S9413	Muenchen	Croatian National Forest
S9200	Muenchen	Croatian National Forest
S9263	Muenchen	Jumping Run Creek
S9171	Muenchen	Croatian National Forest



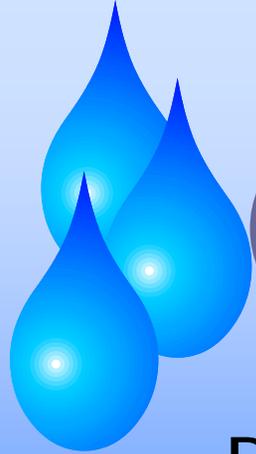
Summary

- Highest prevalence of *Salmonella* were detected from the residential area
- *Salmonella* serotypes and MDR strains found from the watersheds had a public health significance
- Gaminara, Inverness, and Muenchen were predominantly detected from national forest and residential areas



Summary

- All Newport were detected from Open Ground Farm
- ~67% of the isolates showed pan-susceptible
- Highly MDR *Salmonella* strains were detected from watersheds associated with swine farm



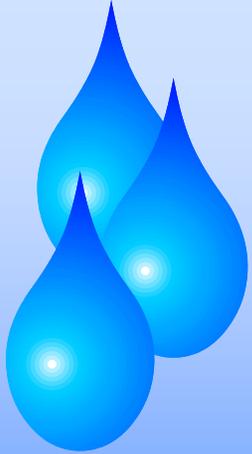
Summary

- PFGE and cluster analysis demonstrated the genetic clonality among *Salmonella* strains recovered from Croatan National Forest and Jumping Run Creek
- Swine strains were cloned and clustered together



Acknowledgements

- USDA CSREES 2004-2007

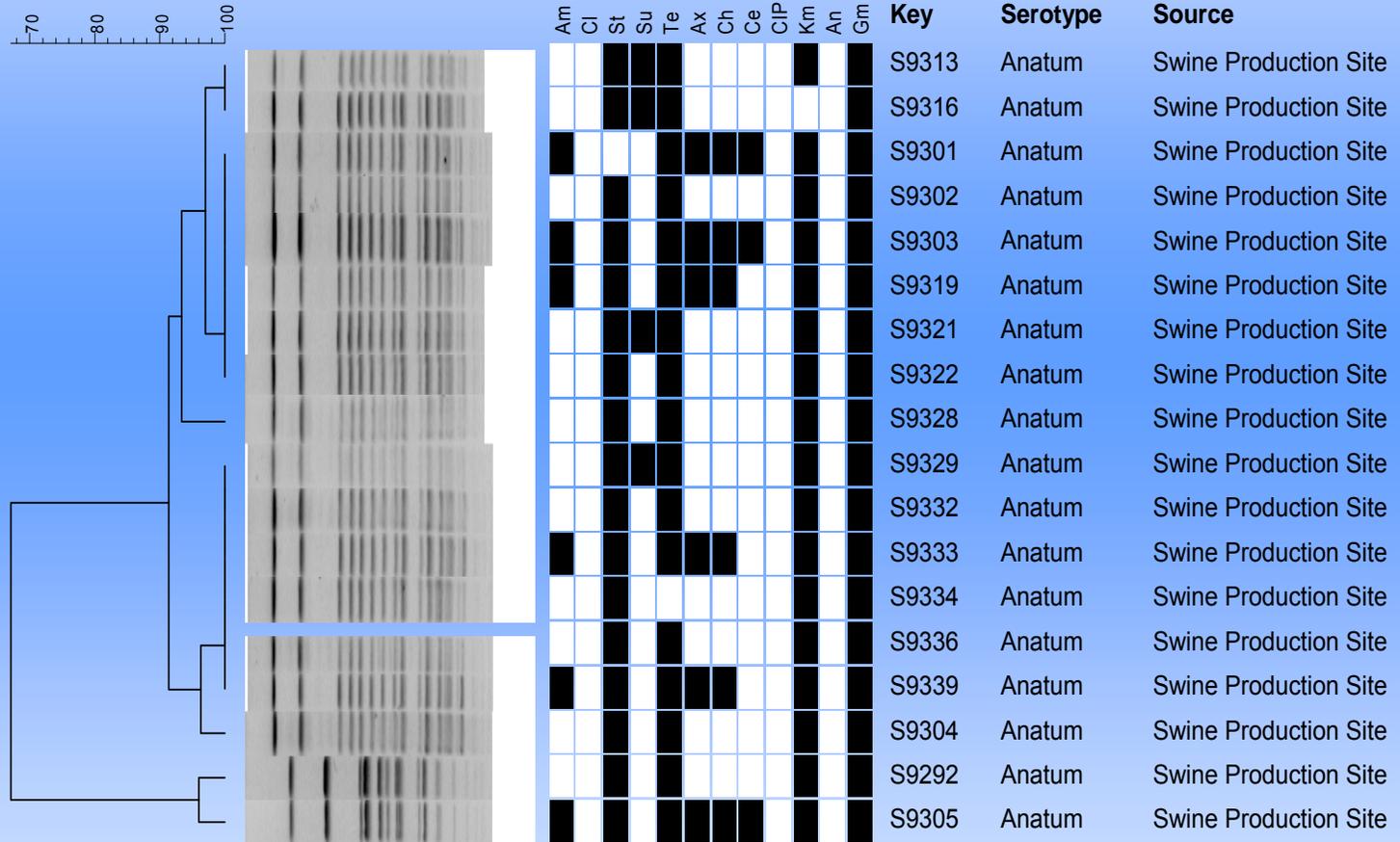


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Salmonella PFGE

Salmonella PFGE

Salmonella R-Type





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Salmonella PFGE

Salmonella PFGE

Salmonella R-Type

